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(71) Applicants and
(72) Inventors: **AMMANNATI, Paola** [IT/IT]; Via E. Zerboglio, 8, I-56125 Pisa (IT). **GIORDANI, Roberto** [IT/IT]; Via V. Nisi, 2, I-56125 Pisa (IT).

(74) Agents: **BARDINI, Marco, Luigi** et al.; Società Italiana Brevetti S.p.A., Corso dei Tintori, 25, I-50122 Firenze (IT).

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(54) Title: ENZYMATIC TREATMENT OF RETINITIS PIGMENTOSA AND RELEVANT PHARMACEUTICAL COMPOSITION IN FORM OF A KIT

(57) Abstract: A kit for the treatment of retinitis pigmentosa containing the enzymes glutathione peroxidase, prolidase, glucose-6-phosphate dehydrogenase and, optionally, aldose reductase in aliquot parts and interactive quantities appropriate for administering said enzymes in accordance with a predetermined time sequence.

WO 2004/030693 A1

TITLE

ENZYMATIC TREATMENT OF RETINITIS PIGMENTOSA AND RELEVANT PHARMACEUTICAL COMPOSITION THEREFOR IN FORM OF A KIT

DESCRIPTION

Field of the invention

The present invention concerns a treatment of retinitis pigmentosa by means of enzymes and a pharmaceutical composition in kit form that can be used for this treatment.

Description of the prior art

Retinitis pigmentosa is a disease of the retina that presents many different pathological manifestations: it may bring about a restriction of the field of vision and create increasing difficulty in adapting to the dark and to penumbra, when it affects the peripheral zones of the retina, which accommodate the greater part of the rod cells that render possible vision in penumbra and the perception of movement in the lateral zones, or may lead to loss of central vision when the cone cells are the ones to undergo modification. The rate of progress of the illness varies from one subject to another. As a general rule, retinitis pigmentosa manifests itself in youth, but often also affects children and acts in an insidious manner.

The causes responsible for this infirmity are as yet unknown and, consequently, there does not exist any cure for those affected by it. The sole certain information at the disposal of experts regards the genetic origin of retinitis pigmentosa, which is in part transmitted by heredity from generation to generation, following mechanisms that are known to geneticists. The greater part of the forms of retinitis pigmentosa are hereditary and three transmission modalities have so far been identified:

of understanding the causes that determine and regulate its course. The lines at present most widely followed by international research are the genetic approach, which seeks to identify the gene or genes responsible for the illness and thus permitting a subsequent intervention by means of modern genetic engineering techniques, the transplant approach, which aims at perfecting a technique that would make possible the transplant of retinal tissue or, at least, the grafting of healthy cells into diseased retinas, and the immunological approach, which sets out to verify some theories that assume the illness to be underlain by some alteration of the immunological system.

The object of the present invention is to provide a pharmacological composition in kit form that will permit retinitis pigmentosa to be efficiently treated.

Another purpose of the present invention is to furnish a method of treating retinitis pigmentosa that will permit a gradual recovery of visual acuity and enlargement of the field of vision, the sharpness of images and the perception of colours and the reconstitution of a normal electroretinogram in the long run.

According to the present invention, these aims are attained by means of the use of particular enzymes for incorporation in a pharmaceutical composition in kit form to be employed for treating retinitis pigmentosa by means of injection into the retrobulbar tissue, all as specified in Claim 1.

More particularly, the enzymes employed are glutathione peroxidase (hereinafter referred to as Enzyme A), prolidase (hereinafter referred to as Enzyme B), glucose-6—phosphate-dehydrogenase (hereinafter referred to as Enzyme C), which are administered in accordance with a

time sequence and modalities to be further specified hereinbelow.

Optionally the treatment may also provide the use of aldose reductase (hereinafter referred to as Enzyme D), which has been found to be useful when - as happens in the greater part of cases - the patient complains of visual fogging.

The enzymes employed in the treatment in accordance with the present invention are available in commerce in lyophilized form and are dissolved in physiological solution to render them available for the treatment.

Each enzyme - in the form of enzyme solution - is administered by means of retrobulbar injection into each eye for three consecutive days, repeating the administration on another two occasions, each separated from its predecessor by a period of one month (for each enzyme). In practice the procedure is as follows:

- one dose of Enzyme A is injected into the retrobulbar tissue of each eye for three consecutive days at the beginning of the treatment, the treatment being then repeated in the second and third month;
- in the fourth, fifth and sixth month one dose of Enzyme B is injected into the retrobulbar tissue of each eye for three consecutive days;
- in the seventh, eighth and ninth month one dose of Enzyme C is injected into the retrobulbar tissue of each eye for three consecutive days;
- if necessary, in the next three months and for three days in each month the treatment is then continued with Enzyme D, the administration mode being exactly as before.

The doses of the various enzymes used at each injection (for each eye) are as follows:

Enzyme A	0.03 - 0.05 U.I.
Enzyme B	5 - 7 U.I.
Enzyme C	7 - 9 U.I.
Enzyme D	7 - 9 U.I.

The preferred doses of the various enzymes at each injection are as follows:

Enzyme A	0.04 U.I.
Enzyme B	6.67 U.I.
Enzyme C	8.00 U.I.
Enzyme D	8.00 U.I.

These doses remain the same for all patients, quite independently of the typology of the alteration. In particular, the enzyme solutions are prepared in such a way that the quantities set out above may be contained within an injection of 0.4 ml. For example, the enzyme solutions suitable for providing injectable doses of 0.4 ml containing the preferred enzyme quantities as set out above are to be prepared as follows

Enzyme A:

Phial containing 10 U.I. of lyophilised enzyme, bring up to 100 ml with physiological solution.

Enzyme B:

Phial containing 1000 U.I. of lyophilised enzyme, bring up to 60 ml with physiological solution.

Enzyme C:

Phial containing 2000 U.I. of lyophilised enzyme, bring up to 100 ml with physiological solution.

Enzyme D:

Phial containing 2000 U.I. of lyophilised enzyme, bring up to 100 ml with physiological solution.

Naturally, these ratios will have to be modified if it is decided to change the dose of any one of the enzymes within its dosing range as set out above.

The enzymes are administered in the order in which they are stated above and the injection cycles are to be continued without interruption.

The kit used for treating retinitis pigmentosa in accordance with the present invention contains the aforementioned enzymes in aliquot parts and interactive quantities appropriate for administering:

a) Enzyme A at a concentration comprised between 0.03 and 0.05 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three consecutive days and for each eye;

b) Enzyme B, starting from the month following the last administration of Enzyme A, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

c) Enzyme C, starting from the month following the last administration of Enzyme B, at a concentration of 7 to 9 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

Optionally, the kit may also contain Enzyme D, to be administered, starting from the month following the last administration of Enzyme C, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

In particular, the enzymes are contained in each kit in lyophilized form and in quantities sufficient for at least one complete series of administrations, each enzyme being subdivided into aliquot parts containing a quantity sufficient for one three-month injection cycle, i.e. eighteen injections, or for one daily administration, i.e.

two injections, and optionally the appropriate doses of physiological solution for constituting said aliquot parts. In particular, in each kit the various enzymes are subdivided into one or more aliquot parts, each of which contains, in the preferred dosing forms as set out above, from 0.04 U.I to 0.72 U.I of Enzyme A, from 6.67 U.I. to 120 U.I. of Enzyme B, from 8 U.I to 144 U.I of Enzyme C and possibly from 8 U.I to 144 U.I. of Enzyme D. Possibly there may also be present three or more aliquot parts of physiological solution from 0.4 to 7.2 ml each.

Patients subjected to the treatment in accordance with the invention found a gradual improvement of visual acuity and field of vision, colour perception and image sharpness (definition). Their electroretinograms improved little by little, eventually becoming reconstituted in the long run. The administered treatment produced a positive response in all the patients, albeit over different periods of time. Follow-up checks after 5-8 years showed that the obtained improvement was permanent and did not bring out side effects of any kind.

Some experimental data confirm the hypothesis that retinitis pigmentosa could be due to an enzyme defect that alters the metabolism of the retina, modifying not only the vision process, but facilitating also the accumulation of pigments, the characteristic feature of the illness.

Studies carried out on rabbits (New Zealand/Fulvo di Borgogna) by one of the applicants showed that the total inhibition of some enzymes causes variations of the electroretinogram, with reduction of the depolarisation wave to the point of extinction and reconstitution of the normal trace after retrobulbar administration of glutathione peroxidase (Enzyme A) and prolidase (Enzyme B). Erythrocyte level reduction of glucose-6-phosphate

dehydrogenase and glutathione peroxidase in patients affected by retinitis pigmentosa was subsequently demonstrated by means of a comparison of their enzyme erythrocyte concentrations with those of subjects not affected by this illness, seemingly in good health, the two groups being homogeneous as regards sex and age.

CLAIMS

1. A pharmaceutical kit for the treatment of retinitis pigmentosa containing the enzymes glutathione peroxidase (Enzyme A), prolidase (Enzyme B), glucose-6-phosphate dehydrogenase (Enzyme C) and, optionally, aldose reductase (Enzyme D) in aliquot parts and interactive quantities appropriate for administering:

a) Enzyme A at a concentration comprised between 0.03 and 0.05 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

b) Enzyme B, starting from the month following the last administration of Enzyme A, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

c) Enzyme C, starting from the month following the last administration of Enzyme B, at a concentration of 7 to 9 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

d) Enzyme D, starting from the month following the last administration of Enzyme C, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

2. A kit in accordance with Claim 1, wherein the concentration of Enzyme A is 0.04 U.I in 0.4 ml of physiological solution, the concentration of Enzyme B is 6.67 U.I. in 0.4 ml of physiological solution and the

concentration of Enzyme C is 8 U.I in 0.4 ml of physiological solution, the concentration of optional Enzyme D being equal to 8 U.I. in 0.4 ml of physiological solution.

3. A kit in accordance with Claim 1 or Claim 2, wherein said kit comprises said enzymes in lyophilised form, in quantities sufficient for at least one series of administrations of from a) to c) and, optionally, also d), subdivided into aliquot parts containing – for each enzyme – a quantity of enzyme sufficient for the constitution of said aliquot parts.

4. A kit in accordance with any one of Claims 1 to 3, wherein said kit comprises said enzymes in lyophilised form subdivided into one or more aliquot parts, each containing from 0.04 U.I. to 0.72 U.I of Enzyme A, from 0.67 to 120 U.I of Enzyme B, from 8 to 144 U.I of Enzyme C and, optionally, from 8 to 144 U.I of Enzyme D, and, optionally, three or more aliquot parts of physiological solution of from 0.4 to 7.2 ml each.

5. Use of the enzymes glutathione peroxidase (Enzyme A), prolidase (Enzyme B), glucose-6-phosphate dehydrogenase (Enzyme C) and, optionally, aldose reductase (Enzyme D) for the preparation of a pharmaceutical composition in kit form for the treatment of retinitis pigmentosa by means of injection into the retrobulbar tissue, said kit containing said enzymes in aliquot parts and interactive quantities appropriate for administering:

a) Enzyme A at a concentration comprised between 0.03 and 0.05 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

b) Enzyme B, starting from the month following the last administration of Enzyme A, at a

concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

c) Enzyme C, starting from the month following the last administration of Enzyme B, at a concentration of 7 to 9 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

d) Enzyme D, starting from the month following the last administration of Enzyme C, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

6. Use of the enzymes in accordance with Claim 6, wherein concentration of Enzyme A is 0.04 U.I in 0.4 ml of physiological solution, the concentration of Enzyme B is 6.67 U.I. in 0.4 ml of physiological solution and the concentration of Enzyme C is 8 U.I in 0.4 ml of physiological solution, the concentration of optional Enzyme D being equal to 8 U.I. in 0.4 ml of phisiological solution.

7. Use of the enzymes in accordance with Claims 5 or 6, wherein said kit comprises said enzymes in lyophilised form, in quantities sufficient for at least one series of administrations of from a) to c) and, optionally, also d), subdivided into aliquot parts containing - for each enzyme - a quantity of enzyme sufficient for the constitution of said aliquot parts.

8. Use of the enzymes in accordance with any one of the preceding claims, wherein said kit comprises said enzymes

in lyophilised form subdivided into one or more aliquot parts, each containing from 0.04 U.I. to 0.72 U.I of Enzyme A, from 0.67 to 120 U.I of Enzyme B, from 8 to 144 U.I of Enzyme C and, optionally, from 8 to 144 U.I of Enzyme D, and, optionally, three or more aliquot parts of physiological solution of from 0.4 to 7.2 ml each.

9. A method for the treatment of retinitis pigmentosa that envisages the administration by means of injection into the retrobulbar tissue of:

a) Enzyme A at a concentration comprised between 0.03 and 0.05 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

b) Enzyme B, starting from the month following the last administration of Enzyme A, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

c) Enzyme C, starting from the month following the last administration of Enzyme B, at a concentration of 7 to 9 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye;

d) optionally, Enzyme D, starting from the month following the last administration of Enzyme C, at a concentration of 5 to 7 U.I. in 0.4 ml of physiological solution for three consecutive days, at monthly intervals, for three months and for each eye.

10. A method in accordance with Claim 9, wherein the concentration of Enzyme A is 0.04 U.I in 0.4 ml of

physiological solution, the concentration of Enzyme B is 6.67 U.I. in 0.4 ml of physiological solution and the concentration of Enzyme C is 8 U.I in 0.4 ml of physiological solution, the concentration of optional Enzyme D being equal to 8 U.I. in 0.4 ml of physiological solution.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/IT 02/00624

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GLORIA E.M.; AMMANNATI P.; SIRAVO D.: "The Pathogenesis of Retinitis Pigmentosa. A Pilot Study on the Clinical Fluoroangiographic and enzymatic effect of Bendazac Lysine" BOLLETTINO DI OCULISTICA, vol. 69, no. 2, 1990, pages 309-318, XP002240485 the whole document	1-10